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Received: 30 March 2021 | Revised: 26 July 2021 | Accepted: 2 August 2021

DOI: 10.1002/ijc.33779

CANCER GENETICS AND EPIGENETICS

SHORT REPORT

Bidirectional Mendelian randomisation analysis of the relationship between circulating vitamin D concentration and colorectal cancer risk

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Abstract

Epidemiological evidence is consistent with a protective effect of vitamin D against colorectal cancer (CRC), but the observed strong associations are open to confounders and potential reverse causation. Previous Mendelian randomisation (MR) studies were limited by poor genetic instruments and inadequate statistical power. Moreover, whether genetically higher CRC risk can influence vitamin D level, namely the reverse causation, still remains unknown. Herein, we report the first bidirectional MR study. We employed 110 newly identified genetic variants as proxies for vitamin D to obtain unconfounded effect estimates on CRC risk in 26 397 CRC cases and 41 481 controls of European ancestry. To test for reserve causation, we estimated effects of 115 CRC-risk variants on vitamin D level among 417 580 participants from the UK Biobank. The causal association was estimated using the random-effect inverse-variance weighted (IVW) method. We found no significant causal effect of vitamin D on CRC risk [IVW estimate odds ratio: 0.97, 95% confidence interval (CI) = 0.88-1.07, $P = .565$]. Similarly, no significant reverse causal association was identified between genetically increased CRC risk and vitamin D levels (IVW estimate β: −0.002, 95% CI = −0.008 to 0.004, $P = .543$). Stratified analysis by tumour sites did not identify significant causal associations in either direction between vitamin D and colon or rectal cancer. Despite the improved statistical power of this study, we found no evidence of causal association of either direction between circulating vitamin D and CRC risk. Significant associations reported by observational studies may be primarily driven by unidentified confounders.

Abbreviations: BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; GWAS, genome-wide association study; IVW, inverse-variance weighted method; LD, linkage disequilibrium; MR, Mendelian randomisation; OR, odds ratio; PRESSO, Pleiotropy Residual Sum and Outlier; SOCCS, the Study of Colorectal Cancer in Scotland.

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Funding information
This work was supported by CRUK programme grant C348/A18927 (M. G. D.). It was also supported by funding for the infrastructure and staffing of the Edinburgh CRUK Cancer Research Centre. E. T. was supported by a CRUK Career Development Fellowship (C31250/A22804). X. Z. is supported by The Darwin Trust of Edinburgh. Y. H. and X. L. was supported by the China Scholarship Council. Y. H. was supported by the Henry Lester Trust and The Great Britain-China Educational Trust. The work received support from COST Action BM1206.

KEYWORDS
causality, colorectal cancer, Mendelian randomisation, vitamin D

What's new?

Vitamin D is associated with outcomes of colorectal cancer (CRC), but a causal relationship hasn't been established. Previous studies could not rule out that predisposition to CRC reduces circulating vitamin D. Here, the authors report results from a bi-directional Mendelian randomization. Using 110 variants and 26,397 patients allowed for a statistically powerful analysis. They found no causal relationship in either direction: low vitamin D did not increase CRC risk, and elevated CRC risk did not reduce vitamin D levels. Previously observed associations, they suggest, may be driven by unknown confounders.

1 | INTRODUCTION

Population-based observational studies consistently demonstrate that lower circulating vitamin D levels are associated with a higher risk of colorectal cancer (CRC).[1] There has been increasing interest in establishing whether this relationship is causal, because it would provide the rationale for exploring dietary vitamin D supplements for CRC prevention. All randomised trials to date, such as the VITAL trial,[2] have been underpowered to detect effects on CRC risk given prohibitive cost and time to observe the required number of incident CRC cases. Mendelian randomisation (MR) is an alternative approach to investigate causality.[3] However, previous two MR studies[4,5] on vitamin D and CRC risk employed no more than six known variants associated with vitamin D, and therefore were hampered by poor genetic instruments (2.84% of vitamin D variance explained) which resulted in inadequate statistical power to detect small to modest causal effects. A recent large genome-wide association study (GWAS) including more than 400 000 Europeans from the UK Biobank cohort identified 143 independent vitamin D-related loci that could explain 5.7% to 10.5% of vitamin D variance,[6] allowing the development of a significantly improved genetic instrument and therefore, a better powered MR study. In addition, previous MR studies only investigated the vitamin D-CRC association, and the null findings therefore could not exclude reverse causation, namely that genetic predisposition to CRC could possibly cause decreased circulating vitamin D concentration. Here, we report a bidirectional MR using much improved genetic instruments to explore whether the observed associations between vitamin D and CRC risk are causal.

2 | MATERIALS AND METHODS

We adopted a two-sample MR approach to estimate causal effects in both directions. To analyse the causal effect of vitamin D on CRC risk, we created a genetic instrument using common variants (minor allele frequency > 5%) identified from a recent GWAS on circulating vitamin D concentration ($P < 5 \times 10^{-8}$) including 417 580 Europeans from the UK Biobank cohort.[6] Effect estimates of these variants along with standard errors (SEs) were extracted from the same study. We then assessed associations between these vitamin D variants and CRC risk by conducting a meta-analysis of effect estimates from 14 GWASs on

TABLE 1 Summarised results of bidirectional Mendelian randomisation study on vitamin D and colorectal cancer risk

		Vitamin D-CRC			CRC-vitamin D	
MR approach	Cases/controls	OR (95% CI)	*P* value	N	β (95% CI)	*P* value
IVW	26 397/41 481	0.97 (0.88, 1.07)	.565	417 580	−0.002 (−0.008, 0.004)	.543
Median based		0.96 (0.84, 1.10)	.569		0.001 (−0.008, 0.009)	.850
MR-Egger		1.03 (0.90, 1.17)	.690		0.016 (0.001, 0.030)	.039
MVMR		0.99 (0.88, 1.10)	.790		−0.002 (−0.008, 0.004)	.539
Stratified IVW						
Colon	4281/24 599	0.91 (0.76, 1.09)	.289	417 580	0.0002 (−0.007, 0.007)	.947
Rectum	3183/24 599	0.80 (0.55, 1.15)	.229		−0.001 (−0.008, 0.006)	.823

Abbreviations: CI, confidence interval; CRC, colorectal cancer; IVW, inverse-variance weighted; MR, Mendelian randomisation; MVMR, multi-variable Mendelian randomisation; OR, odds ratio.

CRC risk (26 397 cases and 41 481 controls),[7] excluding the UK Biobank cohort to control for possible bias introduced by overlapping participants.[8] Statistical power was estimated using the method described by Brion et al.[9] Regarding the reverse causation, we obtained summary effect estimates of CRC-risk variants ($P < 5 \times 10^{-8}$) from two recent meta-analyses of GWASs on CRC risk excluding UK Biobank samples.[7,10] For overlapped variants across the two studies, we retained the effect estimates with smaller *P* values on CRC risk. Effects of these variants on vitamin D concentration were then extracted from the published vitamin D GWAS.[6]

We calculated the R^2 statistic to evaluate linkage disequilibrium (LD) among genetic variants. For any pair of variants in LD ($R^2 > 0.2$), we included the one with the smallest *P* value in relation to the exposure. In an attempt to control for horizontal pleiotropy, we excluded outlier variants using the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) approach.[11] The strength of instruments was assessed by calculating *F*-statistics ($F < 10$ was deemed as a weak instrument).

Causal effect estimates were generated using the inverse-variance weighted (IVW) method, which assumes all genetic variants as valid instruments.[12] We also conducted additional analyses

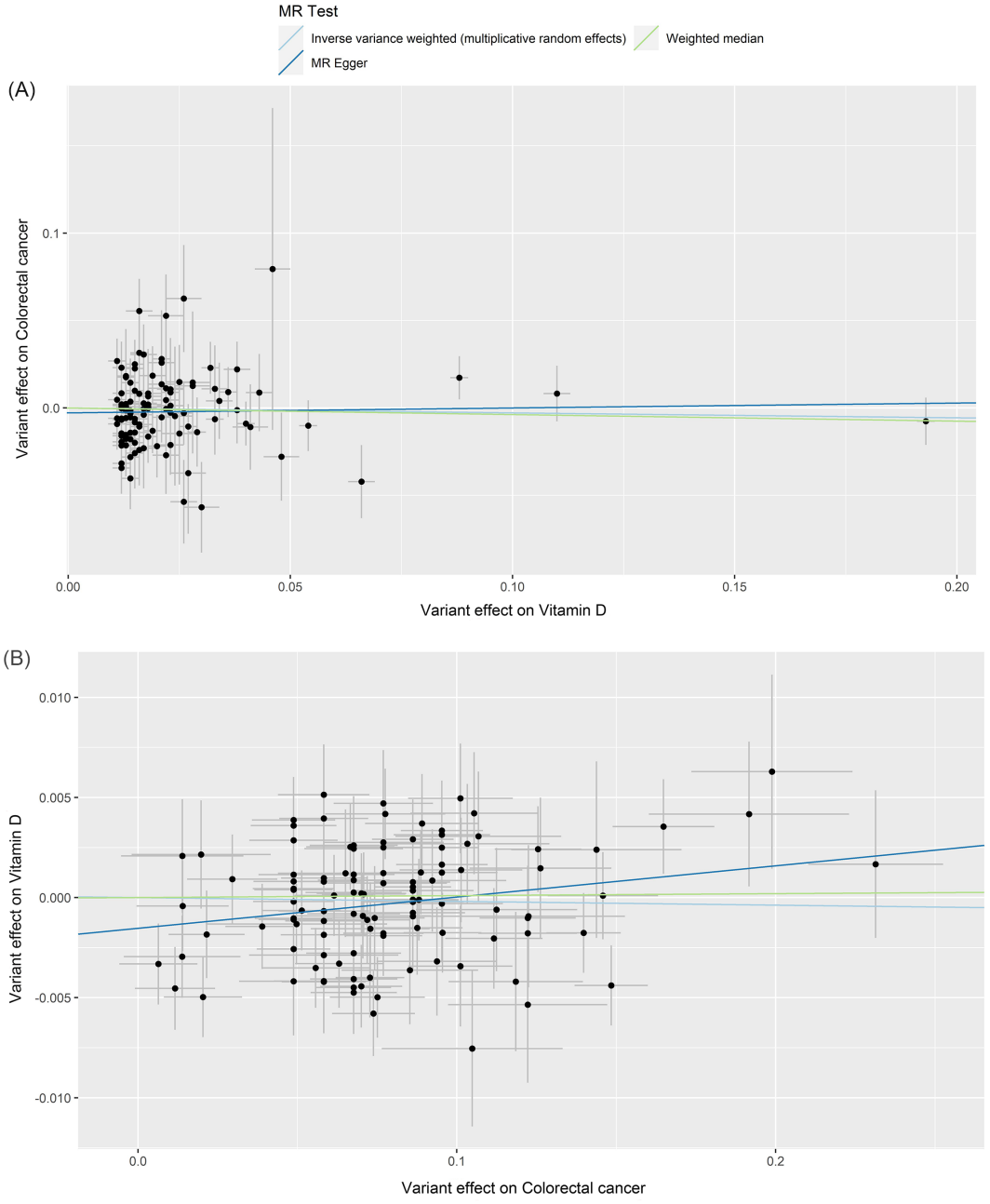

FIGURE 1 Scatterplot of bidirectional Mendelian randomisation analyses [(A) Scatterplot of vitamin D-colorectal cancer risk MR; (B) Scatter plot of colorectal cancer risk-vitamin D MR]. MR, Mendelian randomisation [Color figure can be viewed at wileyonlinelibrary.com]

using the MR-Egger estimator assuming the presence of horizontal pleiotropy,[13] and the median-based estimator assuming half of the genetic variants being invalid instruments.[14] A P value <.008 (Bonferroni corrected threshold) was considered statistically significant. In addition, epidemiological evidence found a potential role of body mass index (BMI) in the vitamin D-CRC association,[15] and previous GWASs identified that vitamin D and CRC risk loci could also be associated with BMI.[6,7,16] Given the possible pleiotropic effect introduced by BMI, we estimated bidirectional causal effects between vitamin D and CRC risk using a multivariable MR approach,[17] leveraging genetic effect of instrumental variants on BMI estimated from the UK Biobank.[18] Previous observational studies have reported differences between the vitamin D effect on colonic cancer and rectal cancer.[19] Thus, we also performed site-stratified IVW MR analysis based on individual-level data of 4281 colon cancer, 3183 rectal cancer cases and 24 599 controls from the Study of Colorectal Cancer in Scotland (SOCCS) and the UK Biobank, and site-specific genetic variants as instruments for reserve causation.[20] All statistical analyses were performed using R (version 3.6.1). Additional descriptions on the study cohorts, including ethics approval, along with technical details of genotyping, quality control and genotype imputation have been published elsewhere.[6,7]

3 | RESULTS

Following LD pruning and QC measures, 110 variants were included as proxies of vitamin D level. Details of genetic variants selection were presented in Figure S1 and basic characteristics along with summary effect estimates of included variants on vitamin D are presented in the Table S1. Using these 110 variants resulted in a strong genetic instrument (assuming 5.7% of vitamin D variance explained), with an F-statistic of 25 241. Based on 26 397 CRC cases and 41 481 controls, the power of the MR analysis investigating the causal effect of vitamin D on CRC risk was 80% for an odds ratio (OR) of 0.91 per SD increase of vitamin D concentration. Summarised results of MR analysis using different estimators are presented in Table 1. Using the IVW method, we identified null causal effect of vitamin D on CRC risk [OR: 0.97 per unit of rank-based inverse-normal transformed vitamin D concentration, 95% confidence interval (CI) = 0.88-1.07, $P = .565$]. Effects of each variant on vitamin D and CRC risk are plotted in Figure 1A. No significant effects were detected using the median-based estimator (OR: 0.96, 95% CI = 0.84-1.10, $P = .569$) and the MR-Egger method (OR: 1.03, 95% CI = 0.90-1.17, $P = .690$). Test of intercept of the MR-Egger estimator showed no significant horizontal pleiotropic effects for the included variants ($P = .220$).

To evaluate any reverse causation effects, a total of 115 variants associated with CRC risk were used as instruments. Effects of these variants on vitamin D concentration were extracted from the previous GWAS and can be found in the Table S2. We found that genetically higher risk of CRC was not significantly associated with the vitamin D concentration of 417 580 participants from the UK Biobank cohort using the IVW estimator (β: −0.002, 95% CI = −0.008 to 0.004, $P = .543$, scatter plot in Figure 1B). Similarly, null causal effects of CRC risk on vitamin D concentration were observed, after Bonferroni correction, using either the median-based estimator (β: 0.001, 95% CI = −0.008 to 0.009, $P = .850$) or the MR-Egger method (β: 0.016, 95% CI = 0.001-0.030, $P = .039$). MR-Egger analysis revealed suggestive evidence for horizontal pleiotropic effects for the variants used as instruments ($P = .011$).

After controlling for possible pleiotropic effects of BMI, our multivariable MR analysis found no significant causal associations between vitamin D concentration and CRC risk in either direction (Table 1). Regarding stratified analysis, we failed to observe any significant causal associations between vitamin D and colon or rectal cancer risk (Tables 1, S3 and S4). A total of 38 variants associated with colon cancer and 26 variants associated with rectal cancer were included as instruments to investigate site-specific reverse causation (Table S5), and we did not observe any significant causal effects of genetically higher risk of colon or rectal cancer on vitamin D level (Table 1).

4 | DISCUSSION

Since circulating vitamin D level is readily modifiable, there has been growing interest in proving causality in the association between vitamin D and CRC risk. Our study is an advance on prior knowledge in the field, where we created a markedly more extensive genetic instrument compared to our previous MRs (110 vs 6 variants). The generation of these genetic instruments, and the largest sample size to date (26 397 cases), has enabled us to conduct statistically powerful analyses. Our findings provide robust evidence that even small-to-modest causal effects of vitamin D on CRC risk are unlikely.

The strength of the genetic instrumental variable and our recent GWAS on CRC risk has also allowed us to perform the first ever bidirectional MR study.[7] This is a novel aspect of considerable importance to understanding whether a genetic predisposition to CRC can cause decreased circulating vitamin D concentration, which was not explored by the previous MR studies. Our results, however, do not support this reverse causation. The null finding of reverse causation suggests that observed associations between vitamin D and CRC in prospective studies are not due to reverse causality, and further questions the role of vitamin D concentration in the biological mechanisms of CRC progression.

In addition to enhanced power, strengths of this study also include that we adopted multiple MR methods such as MR-PRESSO and MR-Egger to detect possible pleiotropic effects of included genetic instruments, which could potentially violate basic MR assumptions.[21] Large sample size, aligned with data granularity, combined with the power of the genetic instruments that we employed, also enabled us to explore the subsidiary aim of understanding the contribution of BMI as a potential confounding, but modifiable effect. After controlling for BMI, our multivariable MR analysis found no significant impact of BMI on vitamin D-CRC risk

association in either direction. To explore the possibility that combining colon and rectal cancer may underestimate the association between vitamin D and a specific cancer location, we conducted a stratified analysis using colon and rectal cancer specific instruments and reran the bidirectional MR analysis. This analysis also confirmed lack of association between vitamin D and either colon or rectal cancer, in either direction. The major limitation of this study is the limited access to individual-level data to aggregate a large enough sample size for further stratified analysis.

In conclusion, this is the first study to present bidirectional assessment of the association between vitamin D and CRC risk using MR. Our findings add to current knowledge by concluding that the observed associations between vitamin D and CRC risk are most likely driven by unknown confounders instead of possible reverse causation or small to modest effects that failed to have been detected by previous MR studies with limited statistical power. Future efforts may focus more on circulating vitamin D level as a predictive biomarker instead of a therapeutic target for CRC prevention.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The individual-level data sets used and/or analysed during the current study are available from the corresponding author on reasonable request. All summary statistics used in this study can be found in Supporting Information.

ETHICS STATEMENT

Study cohorts used were approved by the ethical review board at respective study centres in accordance with the tenets of the Declaration of Helsinki [details: 02/0/097 (NSCCG), 01/0/5 (SOCCS), 05/S1401/89 (GS:SFHS), LREC/1998/4/183 (LBC1921), 2003/2/29 (LBC1936), 17/SC/0079 (CORGI) and 07/S0703/136 (SCOT)]. The UK Biobank was approved by the North West Multi-centre Research Ethics Committee (11/NW/0382).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: He Y, Zhang X, Timofeeva M, et al. Bidirectional Mendelian randomisation analysis of the relationship between circulating vitamin D concentration and colorectal cancer risk. *Int. J. Cancer*. 2021;1-5. doi: 10.1002/ijc.33779